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Light-addressable potentiometric sensor (LAPS) combined with multi-chamber structures to investigate the metabolic activity of cells

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Abstract

LAPS are field-effect-based potentiometric sensors which are able to monitor analyte concentrations in a spatially resolved manner. Hence, a LAPS sensor system is a powerful device to record chemical imaging of the concentration of chemical species in an aqueous solution, chemical reactions, or the growth of cell colonies on the sensor surface, to record chemical images. In this work, multi-chamber 3D-printed structures made out of polymer (PP-ABS) were combined with LAPS chips to analyse differentially and simultaneously the metabolic activity of *Escherichia coli* K12 and Chinese hamster ovary (CHO) cells, and the responds of those cells to the addition of glucose solution.

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Keywords: LAPS, extracellular acidification, chemical imaging, 3D-printed multi-chamber structures, *Escherichia coli* K12, CHO cells ;

1. Introduction

Observing the metabolic activity of living cells under biological circumstances, based on field-effect sensors, can open new doors to study the behaviour of microbiological processes [1-8]. To obtain an accurate result of the

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local activity of cells, precise detection of small changes of the ion concentration is required. LAPS provide a spatially resolved concentration detection of (bio-)chemical species on the sensor surface that is also a suitable sensor system to record chemical images [6, 9]. Multi-chamber structures made out of polymer (PP-ABS) are cost-effective, reproducible, volume-reducing, and easy assembling. A 3D-printing process was applied to create those multi-chamber structures that can be easily attached to the LAPS surface to analyse differentially and simultaneously the metabolic activity of cells (Fig. 1). Exemplarily, the extracellular acidification of *Escherichia coli* K12 for different concentration of cells was evaluated. Furthermore, the metabolic activity of Chinese hamster ovary (CHO) cells, responding to addition of glucose, was recorded.

2. Experimental

2.1. Sensor fabrication

A 540 µm thick, p-doped Si wafer (<100>, 5-10 Ωcm) with a 30 nm SiO_2 layer, grown by thermal dry oxidation, was utilized. The electron-beam evaporation technique was used to bring an additional layer of 30 nm tantalum onto the sensor surface. After the thermal oxidation, a 60 nm thick Ta_2O_5 layer can be achieved which is a good pH-sensitive transducer material with a nearly Nernstian pH sensitivity of 58 mV/pH [10]. In order to create an ohmic contact, the SiO_2 layer on the rear side of the silicon wafer was removed and an aluminum layer with a thickness of 300 nm was deposited. The wafer was diced into chips with the size of 20 mm × 20 mm. Part of the rear-side contact was removed by HF etching to define the area of the LED array, where the light can illuminate the sensor chip.

2.2. Sensor working principle

By applying a positive bias voltage over the LAPS structure with the help of the reference electrodes (Ag/AgCl) (see Fig. 1), a voltage-dependent space-charge region can occur. Electron-hole pairs will be additionally generated within the silicon due to the modulated light illumination from the rear-side (e.g., with the help of LEDs or laser diodes), which results in an external, measurable photocurrent. Due to the pH sensitivity of the LAPS chip, pH variation of the analyte solution on the sensor surface causes a potential shift on the voltage axis of the I/V (photocurrent/voltage) curve. For more details of the LAPS functional principle, see e.g., [1-3].

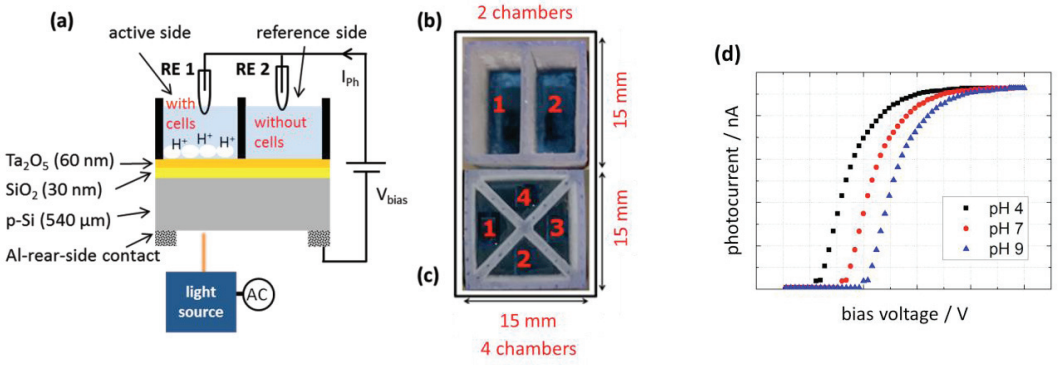

Fig. 1. Schematic illustration of the differential LAPS set-up (a), exemplarily 3D-printed polymer structures attached to the sensor surface with two (b), four measurement chambers (c) and typical I/V curve response to pH changes (d).

2.3. Single-point two-chamber differential measurement setup with Escherichia coli K12

In order to compensate fluctuations of various parameters e.g., pH, conductivity or temperature of the medium, the extracellular acidification of *E. coli* K12 was determined by a single-point differential measurement set-up based on a field-programmable gate array controller (FPGA) [11, 12]. As a first example, 200 µl of bacteria (4.8×10^9 cells/ml, pH 7.4) were pipetted into chamber 1 of the differential set-up (see Fig. 1). In chamber 2, 200 µl of PBS buffer solution was added as a reference. After a conditioning phase, 100 µl of glucose solution (5 mM) was added to both chambers. The differential signal of the pH change of the cell suspension was detected and the acidification rates after adding glucose were calculated (Fig. 2, Table 1).

2.4. Multiple-point four-chamber differential measurement setup with Chinese hamster ovary (CHO) cells

For this experiment, a scanning LAPS set-up was utilized [6, 9, 13]. CHO cells from the cell line CHO-K1 (DSMZ No.: ACC 110) were cultured using cell culture flasks in an incubator. Cells were removed from the cell culture flask using trypsin. The cell suspension was diluted to the desired concentration (4×10^5 cells/ml). A semi-automated image-based cell analyzer (Cedex XS) was used to determine the number of cells in cell solutions. After that, the cells were cultured into the first and second chambers for four hours (Fig. 3) at 37 ˚C in a humidified atmosphere containing 5% CO_2. The culture medium in the first and second chamber were exchanged with 0.4 ml fresh culture medium. Fresh culture media were also filled into the third and fourth chamber, which serve as reference chambers. Four reference electrodes were inserted into the four chambers, respectively, to apply the bias potential to the solution. After exchanging the medium, the first chemical image was directly recorded with a spatial resolution of 500 µm (scanning time: about 10 min). After the recording, 0.1 ml glucose (pH 7 buffer solution, 20 mM) was added into chambers 2 (with cells) and 3 (reference). The chip was left at a room temperature for 5 min, before the second image was recorded.

3. Result and discussion

For the two-chamber measurement, the extra cellular acidification rate of *E. coli* K12 increases with the increase of the cell concentration in suspension. The relationship between the cell number and the acidification rate is not linear (see Fig. 2). According to the higher cell number (4.8×10^9 cells/ml) in the suspension and a constant glucose concentration of 5 mM used in this experiment, the acidification curve reaches a maximum at 2.9 mV/min (see Tab.1). Fig. 3 depicts the differential image of the CHO cell activities caused by adding glucose; As can be seen from the image, additional acidification is only observed for chambers containing CHO cells that consume the added glucose.

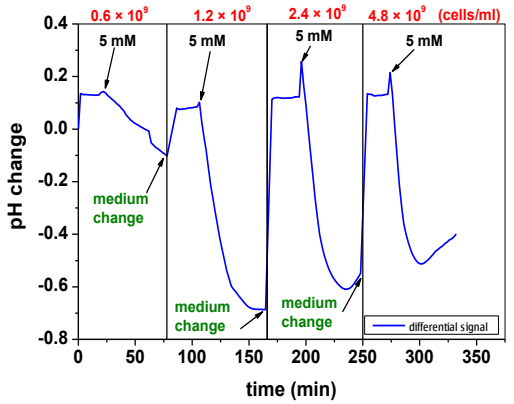

Fig. 2. pH change of four successive measurements with different *Escherichia coli* K12 concentrations after adding 5 mM glucose solution.

Table 1. Acidification rate of *Escherichia coli* K12 for different cell concentrations after adding 5 mM glucose.

cells/ml	acidification rate α (mV/min)
0.6×10^9	(0.2 ± 0.01)
1.2×10^9	(1.7 ± 0.05)
2.4×10^9	(2.4 ± 0.03)
4.8×10^9	(2.9 ± 0.09)

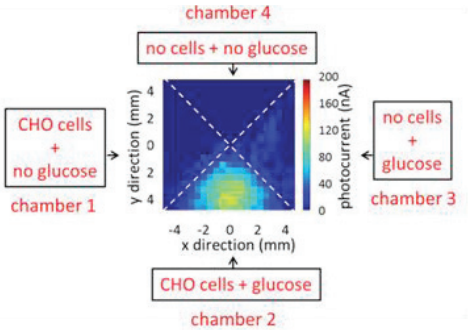

Fig. 3. Differential image of the metabolic activity of CHO cells. Additional acidification is only observed for chamber 2, containing CHO cells and glucose.

4. Conclusion

LAPS sensors combined with new 3D-printed structures were developed to analyse differentially and simultaneously the metabolic activity of living cells. Exemplarily, the extracellular acidification of *Escherichia coli* K12 for different numbers of cells was evaluated. Furthermore, the metabolic activity of Chinese hamster ovary (CHO) cells, responding to the addition of glucose, was recorded. The combination of these two established technologies (LAPS with 3D-printed structures) can enable a promising time- and cost-effective analytical approach to investigate the metabolic activity of living cells in different biological processes.

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